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Scalable ionic gelation synthesis of chitosan nanoparticles for drug delivery in static mixers

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ABSTRACT

The purpose of this study is to synthesize chitosan (CS) nanoparticles (NPs) by ionic gelation with tripolyphosphate (TPP) as crossslinker in static mixers. The proposed static mixing technique showed good control over the ionic gelation process and 152–376 nm CS NPs were achieved in a continuous and scalable mode. Increasing the flow rates of CS:TPP solution streams, decreasing the CS concentration or reducing the CS:TPP solution volume ratio led to the smaller particles. Sylicylic acid (SA) was used as a model drug and successfully loaded into the CS NPs during the fabrication process. Our work demonstrates that ionic gelation–static mixing is a robust platform for continuous and large scale production of CS NPs for drug delivery.

1. Introduction

Chitosan (CS) is a linear copolymer of randomly distributed ß-(1,4)-linked D-glucosamine and N-acetyl-D-glucosamine. Owing to its unique structure, CS possesses numerous attractive features, such as good biocompatibility, biodegradability, permeation enhancing effect, cationic properties, etc. These advantages render CS widely applied in the pharmaceutical and tissue engineering fields. Especially, nanoparticles (NPs) made of CS have been undergoing extensive exploitation for delivery of drugs, proteins/peptides, genes, DNA, etc. (Katas & Alpar, 2006; Krauland & Alonso, 2007; Li, Wang, Peng, She, & Kong, 2011; Makhlof, Tozuka, & Takeuchi, 2011; Saboktakin, Tabatabaee, Maharramov, & Ramazanov, 2010; Xu & Du, 2003; Zhang, Oh, Allen, & Kumacheva, 2004). CS NPs can be synthesized via a number of techniques. These include electrospray, microemulsion, ionic gelation, emulsification solvent diffusion, etc. (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Hamidi, Azadi, & Rafiei, 2008; Liu, Jiao, Wang, Zhou, & Zhang, 2008; Nagpal, Singh, & Mishra, 2010; Songsurang, Praphairaksit, Siraleatmukul, & Muangsin, 2011; Zhang & Kawakami, 2010). Among them, ionic gelation with tripolyphosphate (TPP) as the crosslinker is the mostly adopted technique to fabricate CS NPs, since this method is simple, mild, less toxic and suitable for scaling up. In addition, the size of the achieved NPs can be precisely tuned by adjusting the process parameters, e.g. CS and TPP concentration, CS/TPP weight ratio and volume ratio, pH value, etc. For this method, CS is dissolved in the acidic solution, to which aqueous TPP solution is added. Inter- and intracrosslinking (gelation) of the protonated amine group on the CS molecules with the negatively charged TPP anions occurs spontaneously leading to the formation of the CS particles. By selecting the appropriate conditions, the formed particles can be controlled in the submicron range. Loading a drug into CS NPs can be achieved by addition of the drug to the CS (or TPP) solution prior to the ionic gelation process or adsorption of the drug onto the preformed blank CS NPs. The CS/TPP particles-formation can be divided into the two processes: (1) mixing of the CS and TPP aqueous solution and dispersion of the TPP anions within the CS molecules and (2) crosslinking (i.e. gelation) between the protonated amine groups and TPP anions. These two processes occur in sequence initially but likely to proceed in parallel subsequently, since the CS/TPP complexation is almost instantaneous and will start while mixing is probably still occurring. Therefore, rapid mixing favors the fast and uniform dispersion of the TPP anions within the chitosan chains leading to the formation of the smaller NPs with narrower polydispersity; while slow mixing causes inhomogeneous dispersion of TPP anions resulting in larger particles with wider size distribution. In the literature, only a few studies have described the significance of mixing on the

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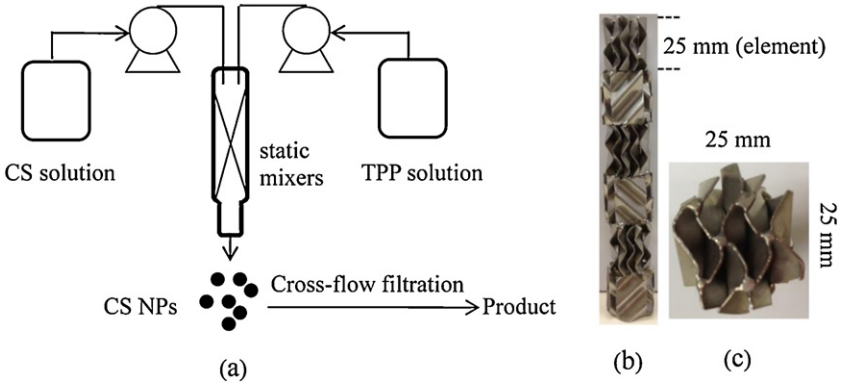

Fig. 1. Scheme of ionic gelation synthesis of CS NPs in static mixers (a), image of 1-segment static mixers composed of 6 elements (b) and top view of static mixers (c).

formation of CS NPs (Nasti et al., 2009; Fan, Yan, Xu, & Ni, 2012). This is probably due to the fact that, most of the reported studies synthesized CS NPs on lab scale. In such a small batch system (e.g. volume from several to tens of milliliter), rapid and uniform mixing can always be achieved by magnetic stirring ensuring the formation of the small NPs. For a large scale production process, however, mixing becomes prominent, as the stirred tank used in the industry is generally characteristic of a slow and inhomogeneous mixing and therefore not a good choice (Dong, Ng, Hu, Shen, & Tan, 2010). Some mixing-intensification equipments are needed for a large scale ionic gelation production of CS NPs. For example, Loh et al. used spinning disc as a process intensification apparatus for mass-production of CS NPs by ionic gelation (Loh, Schneider, Carter, Saunders & Lim, 2010).

Static mixers are one of widely used process-intensification equipments in industry. They are composed of a number of tortuous elements with same configuration. As the flows to be mixed move through the mixers, the tortuous motionless elements continuously convolute and recombine the streams to complete mixing rapidly. A number of additional advantages can also be conferred by static mixers, such as low energy requirement, compact space requirement, low cost, continuous operation, etc. (Thakur, Vial, Nigam, Nauman, & Djelveh, 2003). Our group has used static mixers as process intensification equipment for large scale synthesis of drug nanoparticles and solid lipid nanoparticles (Dong et al., 2010; Dong, Ng, Shen, Kim, & Tan, 2012; Hu, Ng, Dong, Shen, & Tan, 2011). In this work, we aimed to develop a scalable ionic gelation process using static mixers to produce CS NPs in a continuous mode. To our knowledge, synthesis of CS NPs with static mixers has not been reported in literature. Effects of the process parameters, such as number of mixing elements, flow rate, CS concentration and CS/TPP volume ratio on the size of the blank CS NPs (without drug) were explored. Sylicylic acid (SA) was used a model compound and loaded into the CS NPs during the synthesis process for potential intravenous administration. Effect of the drug input on the size and loading efficiency was examined. Morphology of the CS NPs was imaged by field emission scanning electron microscopy (FESEM). Finally, the SA release pattern from CS NPs was assessed in Phosphate Buffer Solution (PBS, pH 7.4).

2. Materials and methods

2.1. Materials

Chitosan hydrochloride (CS) with Mw 200,000–500,000 and deacetylation degree 91.8% was purchased from Xianju Tengwang Chitosan Factory, China. TPP (purity ≥ 98.0%) was supplied by Sigma–Aldrich. The model drug sylicylic acid (SA, purity ≥ 99.0%) was obtained from Sigma. Deionized (D.I.) water was used throughout the study.

2.2. Synthesis of CS NPs in static mixers

Blank and SA-loaded CS NPs were synthesized by ionic gelation in static mixers. SMV DN25 static mixers with a scale of 25 mm were provided by Sulzer Chemtech (Switzerland). Six elements were welded together by being offset at 90° to be one segment (Fig. 1b and c). 1–3 segments were inserted into the glass tube in operation. The detailed scheme of synthesis of CS NPs by ionic gelation in static mixers was illustrated in Fig. 1a. The weight ratio of CS to TPP was kept to be 5:1 throughout the work, as the preliminary results showed that the CS NPs achieved at this ratio were comparatively small and uniform (data not shown). In brief, CS and TPP were dissolved in D.I. water, which were pumped into static mixers by peristaltic pumps. CS NPs were formed spontaneously upon mixing inside static mixers. 300 ml suspension was continuously collected at the exit of static mixers for size and zeta potential measurements. Drug-loaded CS NPs were fabricated in the same way as the blank ones except that 10 or 20 wt% SA (to the weight of CS) was co-dissolved in the CS solution. To remove unloaded drug, the achieved 300 ml suspension was purified with cross-flow filtration (Vivaflow 50, Mw cutoff 100,000) and condensed to 50 ml, which was refilled by D.I. water to 300 ml for repeated cross-flow filtration. Such purification process was performed thrice and 50 ml drug-loaded suspension was achieved finally. A portion of purified SA-loaded CS NPs suspension was directly lyophilized to obtain the powders for drug loading analysis; while a portion of purified SA-loaded CS NPs was lyophilized with 4 times weight sucrose as lyoprotectant to achieve powders for dissolution studies. Some basic process parameters were: (1) the number of mixing elements was 12, (2) the flow rate of CS and TPP solution were 250 and 50 ml/min, respectively, (3) the CS concentration was 0.5 mg/ml and (4) the volume ratio of CS to TPP (CS:TPP) was 5:1. When the effect of one process parameter was examined, the other parameters were kept unchanged unless otherwise described.

2.3. Size and zeta potential (ZP) measurement

Dynamic laser light scattering technique (Nano-Zetasizer, Malvern) was used to measure the size of the CS NPs. This technique first determines the diffusivity of the particles in the suspension based on the time-dependent fluctuations in the intensity of scattered light resulting from the Brownian motion. Size of the particles is thus calculated from the Stokes–Einstein equation. Before measurement, the particles concentration was diluted to ca. 0.25 mg/ml and the measurement was performed at 25 °C. Z-average size and the polydispersity index (PDI) was reported (mean ± SD, $n = 3$). ZP of